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[^{18}F]MK-9470 PET measurement of cannabinoid CB_1 receptor availability in chronic cannabis users

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ABSTRACT

Δ^9-Tetrahydrocannabinol, the main psychoactive component of cannabis, exerts its central effects through activation of the cerebral type 1 cannabinoid (CB_1) receptor. Pre-clinical studies have provided evidence that chronic cannabis exposure is linked to decreased CB_1 receptor expression and this is thought to be a component underlying drug tolerance and dependence. In this study, we make first use of the selective high-affinity positron emission tomography (PET) ligand [^{18}F]MK-9470 to obtain *in vivo* measurements of cerebral CB_1 receptor availability in 10 chronic cannabis users (age = 26.0 ± 4.1 years). Each patient underwent [^{18}F]MK-9470 PET within the first week following the last cannabis consumption. A population of 10 age-matched healthy subjects (age = 23.0 ± 2.9 years) was used as control group. Parametric modified standardized uptake value images, reflecting CB_1 receptor availability, were calculated. Statistical parametric mapping and volume-of-interest (VOI) analyses of CB_1 receptor availability were performed. Compared with controls, cannabis users showed a global decrease in CB_1 receptor availability (–11.7 percent). VOI-based analysis demonstrated that the CB_1 receptor decrease was significant in the temporal lobe (–12.7 percent), anterior (–12.6 percent) and posterior cingulate cortex (–13.5 percent) and nucleus accumbens (–11.2 percent). Voxel-based analysis confirmed this decrease and regional pattern in CB_1 receptor availability in cannabis users. These findings revealed that chronic cannabis use may alter specific regional CB_1 receptor expression through neuroadaptive changes in CB_1 receptor availability, opening the way for the examination of specific CB_1-cannabis addiction interactions which may predict future cannabis-related treatment outcome.

Keywords [^{18}F]MK-9470, cannabis addiction, CB_1 receptor, PET.

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INTRODUCTION

Cannabis is the most commonly produced and consumed illicit drug worldwide. According to the 2013 World Drug Report, the annual cannabis consumption is estimated to be 3.9 percent of the world's population (i.e. 180.6 million of users) aged 15–64 years (United Nations Office on Drugs and Crime (UNODC) 2013). An estimated 10 percent of cannabis users are dependent, and relapse rates for cannabis users in treatment are comparable with those found for other drugs of abuse (Weinstein & Gorelick 2011). Clinical and laboratory studies demonstrated that chronic cannabis smokers can experience withdrawal symptoms upon cessation of cannabis smoking (i.e. insomnia, cognitive impairment, emotional liability, psychiatric depression, irritability and anger) and have difficulty abstaining from cannabis use (Cooper & Haney 2008). Moreover, there is growing evidence that intensive exposure to cannabis increases the risk of adverse health effects, such as impaired respiratory and cardiovascular function, psychotic disorders and a dependence syndrome (Henquet *et al.* 2008; Hall & Degenhardt 2009). However, our knowledge of the effects of long-term cannabis use on the brain remains relatively poor. Therefore, enhanced understanding of the effects of chronic cannabis use on the disruption of interneuronal signalling and information processing may quantify the extent of the risks of long-term use, and

may open the way to novel interventions targeting mediating mechanisms (Murray *et al.* 2007).

Discovery of the endocannabinoid system, comprising endogenous cannabinoids and cannabinoid receptors (Wilson & Nicoll 2002), has prompted interest in understanding the neurobiological effects of chronic cannabis use. The primary psychoactive component of cannabis, Δ^9-tetrahydrocannabinol (Δ^9-THC) (Mechoulam 1970), exerts its known central effects through the activation of the type 1 cannabinoid (CB_1) receptor (Glass, Dragunow & Faull 1997; Iversen 2003). In rodents and humans, CB_1 receptors are highly concentrated in the basal ganglia, hippocampus, cingulate cortex, cerebellum and neocortex (Glass *et al.* 1997), consistent with the major psychological and motor effects of Δ^9-THC (Murray *et al.* 2007). The major mechanism of CB_1 receptor action is an inhibiting modulation of synaptic transmission, by presynaptic action on the release of other neurotransmitters, mainly glutamate, gamma-amino butyric acid and dopamine (Wilson & Nicoll 2002; Hoffman & Lupica 2013), the crucial neurotransmitter for reinforcing and rewarding effects of drugs of abuse. CB_1 receptor expression in the mesocorticolimbic pathway seems to contribute to the rewarding and reinforcing effects of cannabinoids (Cooper & Haney 2008). CB_1-receptor-agonist-induced conditioned place preference and conditioned place aversion are both blocked by administration of CB_1 receptor inverse agonist SR141716A (Braida *et al.* 2004), demonstrating that reward-related effects are mediated by the CB_1 receptor. Administration of SR141716A has also shown to antagonize the positive reinforcing effects of cannabis (Zangen *et al.* 2006). In humans, subjective effects of cannabis such as 'good drug effect', 'high' and 'liking' are also mediated through the CB_1 receptor (Huestis *et al.* 2007).

A number of pre-clinical studies have evaluated the physiological consequences of long-term Δ^9-THC treatment (usually 5–10 days) on CB_1 receptors. These studies have provided evidence that the pharmacological tolerance developed after chronic exposure to cannabis or cannabinoid receptor agonists results in adaptations of CB_1 receptor density (i.e. downregulation and desensitization) (Maldonado 2002; Gonzalez, Cebeira & Fernandez-Ruiz 2005; Clapper, Mangieri & Piomelli 2009) and their downstream cellular effectors (for a review, see Hoffman & Lupica 2013). Decreased CB_1 receptor levels as well as impaired G-protein receptor coupling have been reported in animals made tolerant to the behavioural effects of cannabinoids (Oviedo, Glowa & Herkenham 1993; Rodriguez de Fonseca *et al.* 1994; Sim *et al.* 1996; Romero *et al.* 1998; Breivogel *et al.* 1999; Sim-Selley & Martin 2002). Additional studies have suggested that dysregulated protein phosphorylation downstream of the CB_1 receptor may also contribute to the development of tolerance (Martin, Sim-Selley & Selley 2004). However, the exact role of the CB_1 receptor in the development of dependence and tolerance in humans is still poorly understood. *In vivo* changes in CB_1 receptor availability have been previously described in alcoholic patients (Ceccarini *et al.* 2009; Hirvonen *et al.* 2012b). Recently, we have provided pre-clinical evidence that chronic ethanol exposure leads to decreased CB_1 receptor availability in several areas such as caudate-putamen, hippocampus, and insular and primary sensory cortices (Ceccarini *et al.* 2013a).

Regarding cannabis addiction, a post-mortem investigation of brains from chronic cannabis users found a reduction in [^{3}H]SR141716A binding and decreased CB_1 receptor mRNA levels in the caudate nucleus, putamen, nucleus accumbens and hippocampal cortex (Villares 2007). Concerning *in vivo* investigations, so far there is only one positron emission tomography (PET) imaging study that investigated CB_1 receptor binding in daily cannabis users, demonstrating a selective cortical downregulation of CB_1 receptors (Hirvonen *et al.* 2012a). The aim of the present study was therefore to investigate and replicate *in vivo* changes in CB_1 receptor availability in chronic cannabis users and cannabis-naïve controls using a selective high-affinity CB_1 receptor PET radioligand [^{18}F]MK-9470.

MATERIALS AND METHODS

Participants

The study was approved by the local ethics committee and was conducted in full accordance with the World Medical Association Declaration of Helsinki. All participants provided written informed consent.

Ten chronic cannabis users (age: 26.0 ± 4.1 years) with regular cannabis use (at least once a month for at least 4 years) and 10 cannabis-naïve controls (age: 23.0 ± 2.9 years) were included in the imaging study (Table 1). Cannabis users were recruited from coffee shops in Maastricht (the Netherlands) or via word-of-mouth advertising, and controls were recruited in response to advertisements in the departmental homepage and local community newspapers.

Controls were healthy according to their histories and results of physical examinations, psychiatric screening interviews and routine blood and urine analyses. Cannabis users and controls were excluded if they experienced a history of any major somatic disease or severe mental disorder [meeting DSM-IV criteria (American Psychiatry Association 2000) for psychotic disorder, bipolar disorder, drug addiction disorder (including alcohol) or any other mental disorder requiring treatment by mental health services and/or use of psychotropic medications such as

Table 1 Demographic characteristics for cannabis users and controls.

	Cannabis users	*Controls*	*P*
Gender, male/female	8/2	7/3	> 0.99
Age, years	26.0 ± 4.1	23.0 ± 2.9	0.07
BMI	22.4 ± 2.7	22.9 ± 3.5	0.91
Injected activity of [^{18}F]MK-9470, MBq	145.7 ± 7.4	148.2 ± 5.8	0.44
Age of onset of cannabis use, years	15.8 ± 2.5	—	—
Duration of cannabis use, years	10.2 ± 4.6	—	—
Amount of cannabis, joints per day	2.7 ± 2.4	—	—
Frequency of cannabis use[a] heavy/ moderate/low, *n*	6/3/1	—	
Tobacco smokers/non-smokers, *n*	6/4	0/10	0.03
Number of alcoholic units/day	1.8 ± 1.5	0.9 ± 0.5	0.08

[a]Frequency of cannabis use in the last 12 months assessed by Composite International Diagnostic Interview (CIDI), categorized into 'heavy use' (several times a day), 'moderate use' (from once a day to three to four times a week) and 'low use' (from two to three times a month or less). BMI = body mass index. Data represent mean ± standard deviation.

antidepressants, lithium or antipsychotics], severe head trauma, a family history of psychotic or bipolar disorder according to DSM-IV criteria, a recent history of alcohol abuse (> 5 alcoholic units/day in the past 30 days) to exclude any possible acute-term alcohol effect on CB_1 receptor (Ceccarini *et al.* 'Changes in cerebral CB_1 receptor availability after acute and chronic alcohol abuse and monitored abstinence' J. of Neurosci., in press) and use of any illicit drugs other than cannabis (for cannabis users) in the preceding 2 weeks. Moreover, cannabis users had not experienced negative effects (i.e. cannabis-induced psychosis or psychedelic crisis or bad trip) secondary to cannabis use given the association between the endocannabinoid system (hence the CB_1 receptor) and psychosis (Ceccarini *et al.* 2013b). Participants were asked to abstain from alcohol for 48 hours before PET imaging. Volunteers who were also current tobacco cigarette smokers had a last smoke at least 6 hours before PET.

In addition to the psychiatric evaluation, on the day of PET scanning, blood pressure and heart rate were monitored prior to the scanning sessions. Use of amphetamine, barbiturates, benzodiazepines, cocaine, ecstasy, methamphetamine, morphine, methadone, tricyclic antidepressants and cannabis was also tested prior to the PET scan by urinalysis (MultiDip-Drug Control Screen, Ultimed Products, Ahrensburg, Germany) to ensure that they did not currently abuse drugs other than cannabis. All investigations were evaluated by board-certified specialists.

Image acquisition

Before scanning, all participants were interviewed by a psychiatrist who recorded drug and cannabis consumption parameters (frequency, dose, age of first use) using the Composite International Diagnostic Interview (CIDI, v 1.1) (World Health Organization 1993).

All participants underwent [^{18}F]MK-9470 PET scans. The radiotracer [^{18}F]MK-9470 is an inverse agonist with a high affinity and specificity for the human CB_1 receptor (Burns *et al.* 2007). [^{18}F]MK-9470 was prepared as previously described (Burns *et al.* 2007) and had a radiochemical purity > 95 percent and a specific radioactivity of 150.4 ± 61.5 GBq/μmol.

Cannabis users did not smoke cannabis for 4.0 ± 1.7 days prior to the scanning session to avoid confounding by acute intoxication. All subjects fasted for at least 4 hours prior to the injection of the radioligand to ensure that cerebral radioligand uptake is not influenced by increased serum glucose levels. Before each [^{18}F]MK-9470 administration, subjects were placed with the head placed in a vacuum cushion and the body fixed to minimize head movement. PET data were acquired on a HiRez Biograph 16-slice PET/CT camera (Siemens, Knoxville, TN, USA). Subjects received 146.8 ± 6.7 MBq of [^{18}F]MK-9470 in slow bolus intravenous injection. Images were acquired for 60 minutes (six 10-minute frames) starting 120 minutes post-injection. PET images were reconstructed with 3D-OSEM (ordered-subset expectation maximization) iterative reconstruction including scatter and attenuation correction with a final spatial resolution of 4 mm. Additionally, structural brain abnormalities were evaluated by a standard magnetic resonance imaging (MRI) scan (1.5 Tesla Vision Scanner, Siemens, Germany), both T1-weighted magnetization prepared rapid acquisition gradient echo (3D-MPRAGE) and T2 weighted.

Image processing and statistical analysis

Parametric maps of [^{18}F]MK-9470 binding were expressed on the basis of previously validated quantification

method using the modified standardized uptake value (mSUV) (Sanabria-Bohorquez *et al.* 2010; Van Laere *et al.* 2010, 2012; Gérard *et al.* 2011; Ceccarini *et al.* 2013b). The mSUV index has been shown to be an index for [^{18}F]MK-9470 binding, and was shown to be strongly independent of blood flow (Sanabria-Bohorquez *et al.* 2010). mSUV quantification normalizes radioactivity concentration at each voxel with injected radioactivity dose and subject's weight: mSUV = [activity concentration (KBq/cc) × (subject's body weight (kg) + 70)/2]/injected dose (MBq) (Thie *et al.* 2007). No differences in [^{18}F]MK-9470 plasma concentration and metabolization were found between a subgroup ($n = 4$) of cannabis users that underwent full arterial sampling and controls (see Supporting Information Fig. S1). Therefore, we excluded possible group differences in peripheral metabolism or metabolite-corrected plasma activity input functions that could lead to bias in CB_1 receptor availability determination by the simplified quantification.

The PET data analysis was performed with PMOD v. 2.95 (PMOD Technologies, Zurich, Switzerland) as in our previous CB_1 receptor studies (Van Laere *et al.* 2008, 2009, 2010, 2012; Gérard *et al.* 2011; Ceccarini *et al.* 2013b). For each subject, all PET frames were first realigned for motion correction. The motion-corrected [^{18}F]MK-9470 mSUV individual images were co-registered (rigid body transformation) to the corresponding subject's MRI with a mutual information algorithm, and were then spatially normalized to a specific CB_1 receptor template (Van Laere *et al.* 2008) constructed in Montreal Neurological Institute space (2 × 2 × 2 mm) using non-linear warping. A predefined volume-of-interest (VOI) analysis was performed using an in-house previously created set of VOIs defined on the CB_1 receptor template representing cortical Brodmann areas (BAs) and subcortical grey matter structures (Van Laere *et al.* 2006). Additionally, individual adjustments were made for the subcortical brain areas by delineating these regions manually on the transverse slices of the normalized MRI images. The personalized VOI map was then loaded on the corresponding co-registered and normalized mSUV image, and the average mSUV values within each VOI were then determined using PMOD. mSUV values of cortical BAs were then grouped into larger anatomical brain regions on the base of the number of voxels, and compared between cannabis users and controls using ANOVA and Bonferroni *post hoc* tests ($P < 0.05$), by use of Statistica v. 9.1 (Statsoft Inc., Tulsa, OK, USA).

A voxel-based statistical parametric analysis (SPM2; Wellcome Department of Cognitive Neuroscience, London, England) was also conducted comparing cannabis users and controls in a categorical subject design. Data were first smoothed with a 10-mm full width at half maximum. For statistical assessment of CB_1 receptor availability, non-proportional scaling was used and a relative grey matter analysis threshold of 80 percent of the mean was adopted to exclude extracerebral activity. We also investigated relative CB_1 receptor availability by normalization to the global cerebral mSUV. For analysis of relative CB_1 receptor availability, proportional scaling to the mean voxel value was used. Data were then explored at a voxel-level $P_{height} = 0.005$ (uncorrected) and extent threshold $K_{ext} > 50$ voxels, unless specified otherwise. To exclude the influence of tobacco smoking as confounder, statistical parametric mapping (SPM) analyses were done with and without this parameter as nuisance variable.

RESULTS

Participants

Demographic characteristics of cannabis users and controls are shown in Table 1. There were no differences between the two groups in sex, age, body mass index, injected activity of [^{18}F]MK-9470 and alcohol consumption. Overall, 60 percent of the cannabis users also consumed tobacco.

Table 1 also shows the characteristics of cannabis consumption. Almost all cannabis users had used cannabis on a daily basis, starting from adolescence. The age at first cannabis use was 15.8 ± 2.5 years and the duration of cannabis use was 10.2 ± 4.6 years. For the cannabis consumption, all participants had used cannabis for at least 4 years prior to screening. The frequency of lifetime cannabis use was categorized into 'heavy use' (several times a day; $n = 6$), 'moderate use' (from once a day to three to four times a week; $n = 3$) and 'low use' (from two to three times a month or less; $n = 1$), according to the CIDI (Table 1). Although urine screening for cannabinoids was not positive for all cannabis users (positive for 80 percent), urine cannabinoid results did not have any effect on the change of the CB_1 receptor availability between cannabis users and controls ($F = 1.39$, $P = 0.27$; Supporting Information Fig. S2).

None of the controls had lifetime use of cannabis or any other illicit substance. The number of chronic cannabis users who had used other illicit drugs between 10 and 99 times in their lifetime was low; the great majority of cannabis users (70 percent) were sporadic users (few times per year) of other drugs (including amphetamine, cocaine, ecstasy and opiates). Furthermore, no cannabis user had consumed other drugs in the past 15 days, as confirmed by blood and urine testing.

CB_1 receptor group comparisons

Compared with controls, there was a global decrease of CB_1 receptor availability (−11.7 percent) in the cannabis

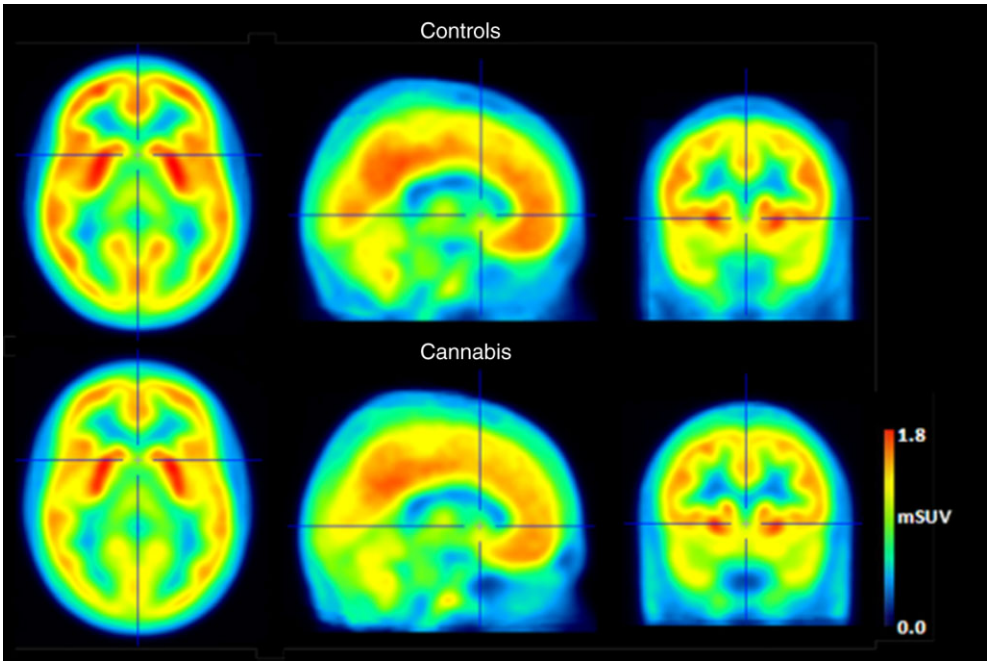

Figure 1 Average modified standardized uptake value (mSUV) parametric images of global CB_1 receptor availability for controls and cannabis users. The colour bar indicates [^{18}F]MK-9470 binding (mSUV) index

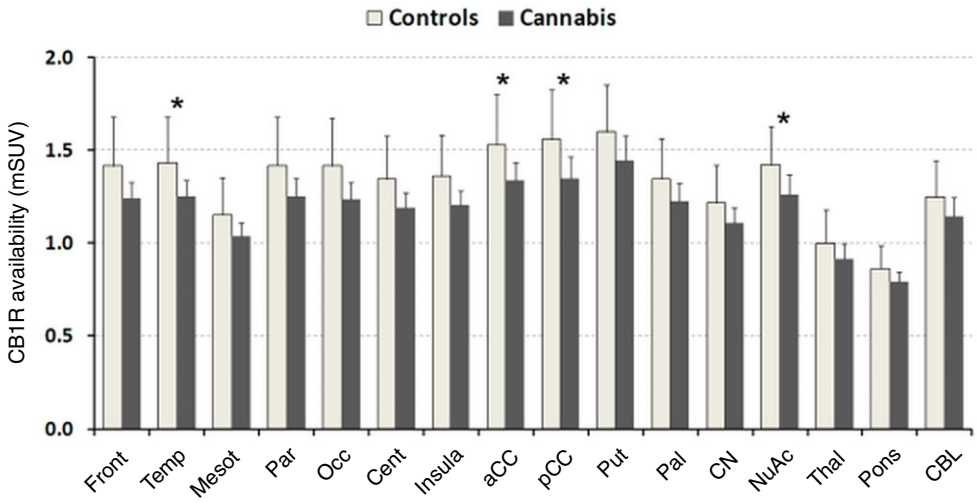

Figure 2 Regional differences in CB_1 receptor (CB1R) availability between controls and cannabis users. Error bars represent one SD. aCC = anterior cingulate cortex; CBL = cerebellum; Cent = central area; CN = caudate nucleus; Front = frontal cortex; Mesot = mesotemporal lobe; NuAc = nucleus accumbens; Occ = cccipital cortex; Pal = globus pallidus; Par = parietal cortex; pCC = posterior cingulate cortex; Put = putamen; Temp = temporal cortex; Thal = thalamus. *$P < 0.05$ ANOVA and Bonferroni *post hoc* tests

group ($mSUV_{CANNABIS} = 1.21 \pm 0.09$, $mSUV_{CONTROLS} = 1.37 \pm 0.24$; $P = 0.06$) (Fig. 1). Regional VOI-based analysis demonstrated that the CB_1 receptor decrease was significant in the temporal lobe (–12.7 percent, $P = 0.04$), anterior and posterior cingulate cortex (for aCC: –12.6 percent, $P = 0.04$; for pCC: –13.5 percent; $P = 0.04$) and nucleus accumbens (–11.2 percent, $P = 0.04$) (Fig. 2 and Table 2), while there was a trend in occipital lobe (–12.8 percent; $P = 0.05$), frontal lobe (–12.2 percent; $P = 0.06$), central lobe (–11.6 percent; $P = 0.06$), insula (–11.5 percent; $P = 0.05$) and parietal lobe (–11.7 percent; $P = 0.08$) (Table 2). In contrast, we did not find decreased CB_1 receptor levels in subcortical brain regions such as mesotemporal lobe, thalamus, putamen, globus pallidum, caudate nucleus, cerebellum and pons ($P \geq 0.10$) (Table 2).

Absolute SPM voxel-based analysis confirmed this bilateral decrease and regional pattern in CB_1 receptor

availability in chronic cannabis users (Fig. 3), with a maximum *t*-value of 3.7 at (x = 48; y = −48; z = 24) in a cluster including superior temporal gyri (BAs 41/39; cluster size K_{ext} = 322]. Relative voxel-based comparison of [^{18}F]MK-9470 binding in cannabis users versus controls revealed a significant decrease in CB_1 receptor availability in six clusters (*t*-value > 3.2) with a maximum *t*-value of 5.1 at (x = −4; y = −76; z = 0; left BA 17), a cluster including occipital regions (cluster size K_{ext} = 447, cluster-level $P_{corrected}$ = 0.028). The other five clusters were located at the left insula (x = 42; y = −4; z = −4; *t* = 4.8), right middle-posterior cingulum (x = 4; y = −44; z = 40; *t* = 4.4), right inferior temporal gyrus (x = 64; y = −22; z = −20; *t* = 4.2), left superior temporal gyrus (x = −52; y = −4; z = −6; *t* = 4.1) and right superior frontal gyrus (x = 26; y = −4; z = 62; *t* = 4.1).

Table 2 Regional CB_1 receptor availability (mSUV) for cannabis users versus controls.

	Cannabis users	*Controls*	*P*
Frontal	1.24 ± 0.09	1.41 ± 0.26	0.06
Temporal	1.25 ± 0.09	1.43 ± 0.25	0.04*
Mesotemporal	1.04 ± 0.07	1.15 ± 0.20	0.10
Parietal	1.25 ± 0.01	1.42 ± 0.26	0.08
Occipital	1.23 ± 0.01	1.42 ± 0.26	0.05
Central	1.19 ± 0.08	1.34 ± 0.23	0.06
Insula	1.20 ± 0.08	1.36 ± 0.22	0.05
aCC	1.34 ± 0.10	1.53 ± 0.27	0.04*
pCC	1.35 ± 0.12	1.56 ± 0.27	0.04*
Putamen	1.44 ± 0.14	1.60 ± 0.25	0.10
Globus pallidus	1.22 ± 0.10	1.35 ± 0.22	0.12
Caudate nucleus	1.23 ± 0.08	1.42 ± 0.20	0.13
Nucleus accumbens	1.11 ± 0.11	1.22 ± 0.21	0.04*
Thalamus	0.91 ± 0.08	1.00 ± 0.18	0.17
Pons	0.79 ± 0.05	0.86 ± 0.13	0.11
Cerebellum	1.14 ± 0.10	1.25 ± 0.20	0.16

*Significant at $P < 0.05$ (ANOVA with Bonferroni *post hoc* correction testing). aCC = anterior cingulate cortex; mSUV = modified standardized uptake value; pCC = posterior cingulate cortex. Data represent mean ± standard deviation.

These findings were independent of tobacco consumption. Cannabis users who also smoke tobacco did not show different CB_1 receptor availability from non-smoking cannabis users in all cerebral regions analysed ($P > 0.05$) (Supporting Information Table S1). SPM results remained when analysis with current tobacco smoking as nuisance variables was done. Moreover, repeated measures ANOVA did not show a main effect of tobacco smoking ($F = 0.66$, $P = 0.42$) or a tobacco smoking per VOI interaction ($F = 0.84$, $P = 0.62$) on mSUV among cannabis users. This suggests that tobacco smoking did not have a significant effect on CB_1 receptor availability as previously demonstrated in animal studies (Gérard *et al.* 2010).

Correlation between CB_1 receptor availability and cannabis consumption

In the cannabis users group, there were no associations between CB_1 receptor availability and the duration of cannabis use or age of onset of cannabis use. We also searched for correlations between CB_1 receptor availability and the level of cannabis consumption categorized into low, moderate and heavy use. Yet, level of cannabis consumption did not influence CB_1 receptor availability observed in cannabis users compared with controls ($F = 1.29$, $P = 0.33$; Supporting Information Fig. S3). However, although we found no significant correlation between CB_1 receptor availability and frequency of

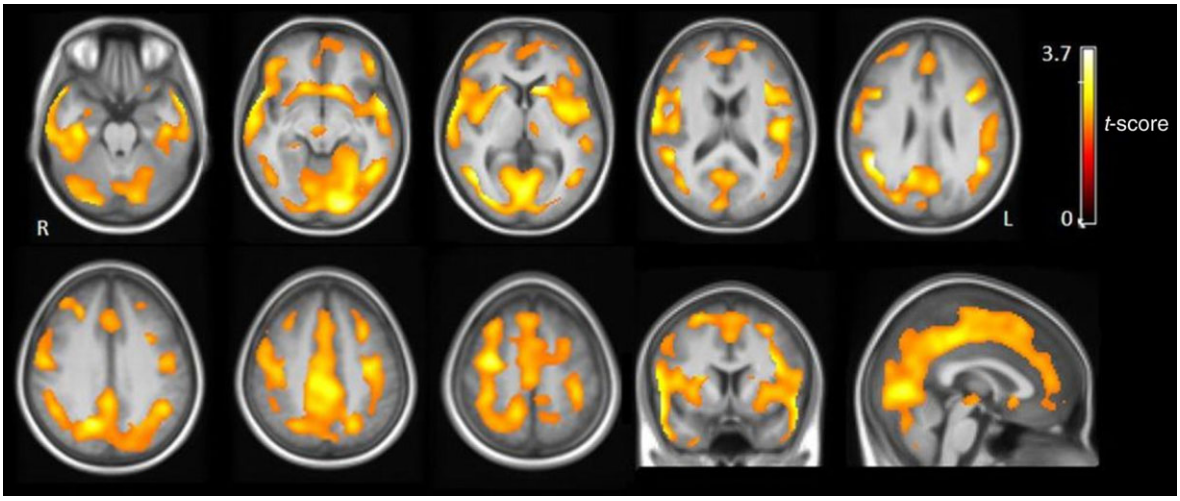

Figure 3 Statistical parametric mapping (SPM) results of the absolute decrease in CB_1 receptor availability in chronic cannabis users compared with controls in transverse, coronal and sagittal sections overlaid on a T1-weighted magnetic resonance imaging template ($P_{height} < 0.005$ uncorrected, $K_{ext} > 50$). The colour bar expresses *t*-score levels

lifetime cannabis use, low cannabis consumption did not produce similar CB_1 receptor downregulation: the subject with the lowest cannabis use showed the smallest global CB_1 receptor decrease (−5.8 percent) compared with cannabis users with a moderate and/or heavy use (−15.8 percent).

DISCUSSION

Pre-clinical, pharmacological and epidemiological evidence has implicated the CB_1 receptor in regulating several Δ^9-THC-induced behavioural effects such as reward, subjective effects and the positive- and negative-reinforcing cannabis effects (Cooper & Haney 2008). However, although the exact role of the CB_1 receptor in the development of dependence and tolerance in humans is still poorly understood, the CB_1 receptor has been suggested as a target for treatment of cannabis dependence (Cooper & Haney 2008; Clapper *et al.* 2009). So far, only one recent PET study has investigated CB_1 receptor availability in chronic cannabis users (Hirvonen *et al.* 2012a). In the present study, we make first use of the selective high-affinity PET radioligand [^{18}F]MK-9470 to investigate *in vivo* measurements of cerebral CB_1 receptor availability in chronic cannabis users compared with control subjects, and to correlate CB_1 receptor changes with cannabis consumption.

Overall, we found a reduction in CB_1 receptor availability in chronic cannabis users compared with cannabis-naïve controls. The decrease in CB_1 receptor availability was significant in the temporal lobe, nucleus accumbens, and anterior and posterior cingulate cortex, and close to significance in several other areas of neocortex as occipital lobe ($P = 0.05$), central lobe ($P = 0.06$), insula ($P = 0.05$), frontal lobe ($P = 0.06$) or parietal lobe ($P = 0.08$). However, we did not find decreased CB_1 receptor levels in subcortical brain regions such as mesotemporal lobe, thalamus, putamen, globus pallidum, caudate nucleus, cerebellum or pons ($P \geq 0.10$). Our results are thus in line with the previous finding of lower [^{18}F]FMPEP-d_2 uptake in chronic cannabis smokers in cortical regions, but not in subcortical brain regions as caudate nucleus, midbrain, thalamus or pons (Hirvonen *et al.* 2012a).

Regional CB_1 receptor downregulation has been demonstrated by both experimental and human studies. With the exception of the nucleus accumbens, no relevant decrease in CB_1 receptor availability was found in the basal nuclei, in the midbrain and in cerebellum. Rodent studies have shown that long-term cannabinoid administration produces an attenuation of both CB_1 receptor availability (downregulation) and CB_1-receptor-agonist-stimulated G-protein activation (desensitization), which are believed to contribute to tolerance to cannabinoid-mediated effects (Romero *et al.* 1997; Breivogel *et al.* 1999, 2003; Sim-Selley & Martin 2002; Martin *et al.* 2004). Decreased CB_1 receptor availability and receptor-mediated G-protein activation have been measured consistently using CB_1 receptor binding in brain sections or membrane homogenates from animals treated with Δ^9-THC, CP55940 or WIN552122, and occurred throughout all CB_1 receptor-containing brain regions (Sim *et al.* 1996; Romero *et al.* 1997; Sim-Selley & Martin 2002). However, the magnitude of downregulation varies, with a smaller change observed in the basal ganglia output nuclei: globus pallidus, entopeduncular nucleus and substantia nigra. The finding of a close regional and temporal correlation between decreases in CB_1 receptor availability sites and decreases in G-protein activation (Breivogel *et al.* 1999; Sim-Selley & Martin 2002) suggests that these two adaptive responses share a common mechanism, such as internalization and degradation of CB_1 receptor.

However, the mechanism by which long-term cannabinoid administration attenuates CB_1 receptor function in the brain has not yet been resolved. The observed decreased CB_1 receptor availability may also be the consequence of other molecular mechanisms such as cross-desensitization, allosteric modulation, disruption in CB_1 receptor dimerization and trafficking. By analogy with cultured cell models, the mechanisms of CB_1 receptor desensitization and downregulation might include G-protein coupled receptor kinase-mediated phosphorylation and association of the receptor with β-arrestin, although this remains to be established in the central nervous system. Changes in expression of the gene encoding the CB_1 receptor might also play a role in some brain regions, but not others. Another cellular consequence of chronic cannabinoid administration is a compensatory increase in cyclic adenosine monophosphate synthesis with concomitant enhancement of protein kinase A activity (Martin *et al.* 2004). However, from the current findings, it remains unclear whether changes in receptor availability are due to altered receptor density, or changes in receptor affinity or in receptor trafficking, as these mechanisms cannot be distinguished with PET. However, previous studies demonstrated significant CB_1 receptor downregulation as a result of loss of CB_1 receptor protein after chronic agonist exposure (Sim-Selley 2003; Sim-Selley *et al.* 2006), suggesting a change in receptor density rather than affinity. Nevertheless, the decreased CB_1 receptor availability cannot be due to a possible competition effect of Δ^9-THC with the [^{18}F]MK-9470 radioligand. Firstly, Δ^9-THC acts non-selectively as partial agonist at both cannabinoid CB_1 and CB_2 receptors (Pertwee *et al.* 2010), whereas the CB_1 receptor subtype selectivity of [^{18}F]MK-9470 is about 60-fold over CB_2 receptors (Burns *et al.* 2007). Secondly, the affinity of

Δ^9-THC is similar to that of the endocannabinoid anandamide (Pertwee *et al.* 2010). Comparing the affinity of the endocannabinoids (26–209 nM for AEA and even above 10 μM for 2-AG) to the nanomolar affinity of [^{18}F]MK-9470 (0.7 nM) for the human CB_1 receptor, it is rather unlikely that Δ^9-THC competes with the radioligand [^{18}F]MK-9470 for binding to the CB_1 receptor. To look at the status of a receptor without any influence from the endogenous or exogenous ligand, high-affinity radioligands are necessary, as is the case with [^{18}F]MK-9470. Furthermore, as demonstrated previously for [^{11}C]MePPEP, a CB_1 receptor radioligand with a similar affinity, current high-affinity CB_1 receptor radioligands are not displaceable *in vivo* by high doses of endogenous agonist or synthetic agonists (Terry *et al.* 2008).

Animal and human studies furthermore reported that decreased CB_1 receptor availability recovered after cannabis abstinence of at least a few weeks (Sim-Selley *et al.* 2006; Hirvonen *et al.* 2012a). Considering the similar demographic and cannabis history characteristics of the cannabis users group analysed in the current study with the one investigated by Hirvonen *et al.* (2012a), and considering that after 4 weeks of continuously monitored abstinence from cannabis CB_1 receptor density returned to normal levels (Hirvonen *et al.* 2012a), decreased CB_1 receptor binding may thus reflect a state condition rather than an enduring trait-like feature. However, longer follow-up is necessary to differentiate this.

Interestingly, our findings confirm that chronic cannabis consumption decreases CB_1 receptor availability in cortical regions but also in the nucleus accumbens, a key structure for reward and drug reinforcement (van der Stelt & Di Marzo 2003). The brain circuitry that is shared between cue-, drug- and stress-primed reinstatement converges on the anterior cingulate cortex and has a final common output through the nucleus accumbens (Kalivas & McFarland 2003). Moreover, it has been reported that glutamatergic projections from the prefrontal areas to the nucleus accumbens play an important role in relapse behaviour (Kalivas & McFarland 2003). Nucleus accumbens has been also identified as one of the two brain trigger zones for stimulant and rewarding effects of Δ^9-THC, together with the ventral tegmental area (VTA) (Zangen *et al.* 2006). Briefly, microinjections of Δ^9-THC into the posterior VTA or into the shell of the nucleus accumbens increased locomotion, and rats learned to lever press for injections of Δ^9-THC into each of these regions, an effect that is blocked by SR141716A (Zangen *et al.* 2006). Moreover, repeated exposure to Δ^9-THC resulted in tolerance to the inhibitory effects of WIN55,212-2 in the nucleus accumbens, and the tolerance was associated with a loss of endocannabinoid-mediated long-term depression (Hoffman *et al.* 2003). It seems that either short-term or long-term exposure to Δ^9-THC can limit the degree to which nucleus accumbens glutamate synapses undergo long-term depression, and this appears to result from a downregulation of CB_1 receptor function and the ability of endocannabinoids to initiate this form of synaptic plasticity (Hoffman & Lupica 2013). This effect of Δ^9-THC may alter motivational processes mediated by the nucleus accumbens and may play a role in modulating the reinforcing properties of other abused drugs acting either directly within the nucleus accumbens or indirectly by altering dopamine function in this brain structure (Hoffman & Lupica 2013).

Notably, our findings did not show any correlations between CB_1 receptor availability and age at onset of smoking, number of joints per day or duration of cannabis use. One possible reason could be the small variance in these variables for the current population that might have obscured some of the possible correlations. However, the subject with lowest cannabis use showed the smallest global CB_1 receptor decrease (−5.8 percent) compared with the cannabis users with moderate or heavy use (−15.8 percent; Supporting Information Fig. S3). This is in line with Hirvonen and colleagues, who reported that the cannabis users who, on average, smoked three joints per day showed a smaller global CB_1 receptor decrease (~12 percent) in comparison with cannabis users who smoked 10 joints per day on average (~20 percent) (Hirvonen *et al.* 2012a). This latter study furthermore revealed a relationship between decreased CB_1 receptor density and years of cannabis smoking. Considering that subjects who occasionally smoke cannabis do not develop tolerance to the level that heavy chronic daily smokers do (Cooper & Haney 2008), and that occasional cannabis users likely differ in terms of CB_1 receptor downregulation, we could therefore assume that CB_1 receptor downregulation contributes to the development of tolerance of cannabis. Further studies need to address this issue by comparing CB_1 receptor availability in heavy, moderate and occasional cannabis smoking.

Our findings should be interpreted considering some potential limitations. Firstly, cannabis users were scanned on average after 5 days of cannabis abstinence to avoid acute intoxication. This is different from Hirvonen *et al.* (2012a) where cannabis smokers were imaged on the day following the last cannabis consumption to maximize the effect size of the hypothesized CB_1 receptor downregulation. Thus, a potential abstinence effect due to short-term deprivation of cannabis cannot be fully excluded. However, as results are very similar to Hirvonen *et al.*, such effect seems to be modest at most. Secondly, for quantification of CB_1 receptor availability, the simplified mSUV measure was used. Modelling of tracer kinetics indicated that the use of mSUV as quantification measure is an index of CB_1 receptor availability

related to distribution volume V_T (Sanabria-Bohorquez *et al*. 2010). Moreover, it has been recently demonstrated that this approach allows a more practical acquisition protocol in patients and is a valid simplified quantification providing an index of tracer binding, given that no group differences in peripheral metabolization are present (Van Laere *et al*. 2010, 2012; Gérard *et al*. 2011; Ceccarini *et al*. 2013b). The latter was also explicitly demonstrated for the subjects in this study. Finally, because of the small sample size limited to 10 cannabis users, the decreased CB_1 receptor availability found in the current study awaits independent replications in larger samples. However, considering the similarity between the current findings with the ones obtained with 30 cannabis users (Hirvonen *et al*. 2012a), we think that, overall, our main conclusions should be considered well founded rather than preliminary.

In conclusion, the results presented here provide further *in vivo* evidence that chronic cannabis use produces regional downregulation of CB_1 receptors. Future studies should focus on longitudinal CB_1 receptor evaluation after episodes of cannabis deprivation in order to understand the state versus trait differences that might open the way for the examination of specific CB_1-cannabis addiction interactions, which may predict future cannabis-related treatment outcome. Regarding the current pharmacological treatments of cannabis dependence, a range of medications have been tested in a human laboratory model of cannabis dependence as oral Δ^9-THC, divalproex, bupropion, nefazodone, buspirone, dronabinol and naltrexone (Weinstein & Gorelick 2011). Abstinence in cannabinoid-dependent subjects elicits withdrawal symptoms that promote relapse into drug use, suggesting that pharmacological strategies aimed at alleviating cannabis withdrawal might prevent relapse and reduce dependence (Clapper *et al*. 2009). However, there are currently no medications approved by any national regulatory authority to treat cannabis-related disorders. The only treatment currently shown consistently to alleviate cannabinoid withdrawal in both animals and humans is substitution therapy using oral Δ^9-THC (Weinstein & Gorelick 2011). However, new genetic and pharmacological tools are available to increase endocannabinoid levels by targeting fatty acid amide hydrolase (FAAH) or monoacylglycerol lipase (MAGL), the enzymes responsible for the degradation of the endogenous cannabinoid ligands anandamide and 2-arachidonoylglycerol, respectively. In line with this consideration, a pre-clinical study has shown that acute administration of either FAAH inhibitor URB597 or MAGL inhibitor JZL184 significantly attenuated rimonabant-precipitated withdrawal signs in Δ^9-THC-dependent mice (Schlosburg *et al*. 2009), supporting the concept of targeting endocannabinoid-metabolizing enzymes as a promising treatment for cannabis withdrawal.

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Conflict of Interest

There is no conflict of interest to declare.

Authors Contribution

The authors JC, RK and KVL contributed to the study concept and design. JC and DK were involved in data acquisition. RK, JVO, CH and KVL provided comments on the draft. All authors critically reviewed content and approved the final version of the manuscript for publication.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1 Comparison of tracer metabolization and metabolite-corrected arterial input function between controls and cannabis users

Figure S2 Global grey matter decrease of CB_1 receptor (CB1R) availability in cannabis users (CAN) compared with controls (CON) versus urine cannabinoid results

Figure S3 Global grey matter decrease of CB_1 receptor (CB1R) availability in cannabis users (CAN) compared with controls (CON) versus cannabis consumption

Table S1 Regional CB_1 receptor availability (mSUV) for tobacco smokers versus nonsmokers cannabis users